Clinical studies and tissue analyses in the earliest phases of rheumatoid arthritis: In search of the transition from being at risk to having clinically apparent disease

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ENGLISH SUMMARY

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by pain, swelling and stiffness of the joints due to synovial inflammation, which is the hallmark of the disease. It is a debilitating chronic erosive disease that affects 1-2% of the population worldwide. Several risk factors associated with RA have been recognized, but the etiology is largely unknown. Moreover, the factors leading to the onset of synovial inflammation are unknown.

In this thesis clinical studies (part I) and analyses of the synovium (part II) and the immune response in different compartments (part III) were performed in the earliest phases of RA to get more insight into the mechanisms behind the transition from a phase during which individuals are at risk for developing RA, characterized by systemic autoimmunity associated with RA, to having clinically apparent disease.

I. CLINICAL ASPECTS DURING THE EARLIEST STAGES OF RA

RA is a heterogeneous disease, both on a clinical and a molecular level. Together with the absence of a diagnostic test RA patients are therefore classified for research purposes based on mostly clinical features, according to the 1987 American College of Rheumatology (ACR) criteria for RA. These criteria lead to fulfillment of the criteria during a late stage of the disease. To facilitate the study of persons in an earlier disease stage and to be able to start early and effective intervention, new criteria were developed by a joint working group of the ACR and the European League Against Rheumatism (EULAR): the 2010 ACR/EULAR criteria for RA. In chapter 2 we showed that 1) use of the 2010 ACR/EULAR criteria clearly allows earlier diagnosis of RA, 2) RA according to the 2010 ACR/EULAR criteria is less severe than RA according to the 1987 ACR criteria, and 3) the disease heterogeneity has increased in RA according to the 2010 ACR/EULAR criteria. How this will affect clinical trials and whether more strict in- or exclusion criteria should be used or stratification by individual criteria is required needs to be addressed in future studies. Moreover, we and others have observed that by application of the 2010 ACR/EULAR criteria some patients with self-limiting disease may be falsely diagnosed with RA, but that, on the other hand, unclassified arthritis (UA) patients may develop erosive disease as well. This suggests that there is a sustained need for prediction models for the development of persistent and erosive disease in early arthritis patients, which may be different from fulfillment of classification criteria for RA.

Besides the importance to be able to predict outcome in patients who already have arthritis, it is important to determine factors predictive and associated with RA in individuals who are at increased risk of developing the disease. This will provide insight into the mechanisms behind the development of RA and could offer more clarity about the risk of transition to RA in individual subjects. Moreover, accurate prediction of development of RA may, ultimately, help to determine who may need preventative treatment. Several risk factors for RA are known, but so far most of them were investigated in association studies. In chapter 3 we showed for the first time in a prospective cohort of autoantibody-positive individuals that smoking and overweight increase the risk of development of RA, but the underlying biological mechanisms are as yet not well understood. Citrullination
of peptides in the lungs or gingiva due to smoking may play a role and both smoking and overweight may be involved in driving low-grade systemic inflammation. Our results show the importance of modifiable factors in the development of RA, which should be critically evaluated in future clinical research aimed at disease prevention.

II. THE SYNOVIUM DURING THE EARLIEST STAGES OF RA

Studying the target tissue of RA, the synovium, is highly important to get more insight into the disease pathogenesis. Mini-arthroscopy allows synovial biopsy sampling with low complication rates, which is performed in a limited number of research centers. It is sometimes seen as a procedure that is quite invasive and therefore not easily implemented in clinical trials or basic research projects. In chapter 4 we investigated expectations and experience of patients undergoing mini-arthroscopic synovial biopsy sampling in comparison to MRI for studying synovial inflammation. We observed that mini-arthroscopy is well tolerated compared to MRI, both in early arthritis patients and in autoantibody-positive individuals without arthritis who are at risk for the development of RA. These results refute the belief that mini-arthroscopy is not well tolerated and support the use of mini-arthroscopy in a research setting, which may increase options for collaborations between multiple research centers.

In chapter 5 we performed MRI and mini-arthroscopic synovial biopsy sampling to analyze the synovial tissue of autoantibody-positive individuals who are at risk of developing RA. Our aim was to evaluate whether subclinical synovitis precedes the onset of clinically apparent RA and to get more insight into the pathways involved in the onset of arthritis. We did not find clear cut synovial inflammation before the development of clinically apparent arthritis, suggesting that systemic autoimmunity precedes synovial cellular infiltration. Interestingly, we did find that subtle synovial infiltration by T cells may precede the onset of RA, but this needs further validation.

Since we did not observe clear cut signs of synovial inflammation in these autoantibody-positive individuals before onset of arthritis, whereas most of them suffer from arthralgia, we hypothesized that arthralgia could perhaps be caused by the presence of molecules in the synovium able to induce pain. One of the major candidate pathways involved in arthralgia is the prostaglandin (PG) E₂ pathway, which is also known for its pro-inflammatory characteristics. In chapter 6 we could not find clear evidence that arthralgia in the earliest phases of arthritis can be explained by altered synovial expression of enzymes of the PGE₂ pathway. Pain in these patients may therefore be regulated by other pathways or originate at sites other than the synovium. In addition, we observed increased synovial expression of PGE₂ enzymes in early spondyloarthritis (SpA) patients compared to RA and UA patients, supporting previous studies suggesting involvement of the PGE₂ pathway in the pathogenesis of SpA.

III. THE IMMUNE RESPONSE IN DIFFERENT COMPARTMENTS DURING THE EARLIEST STAGES OF RA

In chapter 5 it was suggested that synovial tissue infiltration is a process relatively late in RA pathogenesis and happens (sub)acutely. This hampers the study of the synovium
during the transition phase. Since the immune reaction in lymph nodes generally precedes the influx of effector cells into the target tissue, analysis of lymph node tissue may gain new insights into the earliest phases of RA pathogenesis and in the initiation of synovial infiltration. Therefore we initiated lymph node biopsy sampling in different phases of RA. In chapter 7 we described the technique of core-needle biopsy sampling of inguinal lymph nodes and we introduced it as a new research tool in RA. This technique provides lymph node biopsy samples of good quantity and quality for different analysis methods. Importantly, we showed that the procedure was generally well tolerated and that, other than a small hematoma requiring no therapy in most of the cases, no major complications were reported. This may facilitate research of lymph node tissue in other centers. In chapter 8 we investigated the cellular composition of lymph node biopsies of autoantibody-positive individuals in comparison to healthy controls and early arthritis patients. We observed an increase in activated CD8 positive CD69 positive T cells in early arthritis patients compared to healthy controls. In addition, we observed an increase in CD19 positive B cells in early arthritis patients compared to healthy controls and a similar trend for the autoantibody-positive individuals compared to healthy controls. These studies are in line with observations in animal models of arthritis, although further validation of these results is necessary. In addition, it will be important to evaluate whether individuals who will develop RA over time can be discriminated from those who will not and to determine which pathways are involved in the transition to RA.

In chapter 9 we investigated the T-cell receptor (TCR) repertoire in peripheral blood and synovial tissue of early and established RA patients to evaluate the possible role of expanded T-cell clones in the pathogenesis of RA. We observed highly expanded T-cell clones in the synovium of early RA patients, but not in their peripheral blood. These highly expanded synovial T-cell clones were significantly higher expanded in early RA compared to established RA patients. In addition, we showed that between different joints there was considerable overlap in specific TCR sequences of highly expanded T-cell clones, which was not the case in synovial tissue paired with peripheral blood samples. Together, this suggests that in early RA clonally expanded T cells may be retained in the synovium, possibly as a response to the presence of specific (auto)antigen(s) in the synovium. To further extend these analyses it would be necessary to characterize the expanded T-cell clones in detail with the ultimate goal to be able to discover RA-specific antigens.

Overview

In Figure 1 the observations in this thesis are graphically depicted within the framework of existing knowledge about the earliest phases of RA. In this thesis we have shown in a prospective study that smoking and overweight contribute to the development of RA 1). This may be due to promoting low-grade systemic inflammation or more specific, for example by citrullination of peptides in the lungs. 2) Autoantibodies against these citrullinated peptides (ACPA) and other RA-specific autoantibodies (RF) may at some time point be detected in the peripheral blood of these individuals. From then on these individuals are classified as individuals having an increased risk for the development of RA by the presence of systemic autoimmunity associated with RA. 3) In this thesis we
have shown that in these individuals activation of B cells may occur in the lymph nodes. 4) However, we did not find clear cut signs of synovitis months before the development of clinically manifest arthritis, suggesting that these lymph node changes occur before onset of synovitis. We could not clearly explain arthralgia in this phase of the disease by synovial involvement of the \( \text{PGE}_2 \) pathway, a major pain pathway. Pain may therefore be regulated by other pathways or originate at sites other than the synovium. 5) In patients who just developed RA, we observed highly expanded T-cell clones in the synovium specifically, suggesting that specific T cells are retained or proliferate in the synovium. In addition, we observed that activated T cells and B cells were increased in lymph nodes of early arthritis patients. 6) Patients who have developed arthritis may or may not (yet) fulfill classification criteria for RA. In this thesis we have shown that with the application of the newly developed 2010 ACR/EULAR criteria more patients are classified as RA and in an earlier phase than with application of the 1987 ACR criteria. However, a subset may be false negatively or false positively classified. 7) Of note, in addition to diagnostic criteria, there is still a need for prediction models for long term outcome.

**Figure 1.** A summary of the observations in this thesis within the framework of existing knowledge about the earliest phases of RA. Note1: Observations in this thesis in bold print. Note2: Specific phases up to the development of rheumatoid arthritis in Italic print. Note3: the numbers do not imply chronological order of occurrence of described events, but are merely used to refer to the description in the text. RF: rheumatoid factor, ACPA: anti-citrullinated protein antibodies, RA: rheumatoid arthritis.